DNA Profiling & Its Role in Forensic Odontology

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Abstract

The modern advances in DNA profiling have made DNA evidence more widely accepted in courts. This has transmuted the aspect of forensic odontology. DNA profiling/DNA fingerprinting has moved a long way from the conventional fingerprints. DNA that is responsible for all the cell's activities, yields valuable information both in the healthy and diseased individuals. When other means of identification become impossible following mass calamities or fire explosions, carbonization and dissolution, teeth contain a rich source of DNA as they have a high chemical and physical resistance. The recent advancement in the isolation of DNA and the ways of running a DNA fingerprint are highlighted in this literature review

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Introduction

Identification of disaster victims involves comparing ante-mortem data available with their postmortem reports.

When ante- mortem data are unavailable, DNA profiling/ fingerprinting becomes the only and reliable method for identification. Dental pulp contain a rich source of DNA.[1] Identifying the deceased is not only important for the family but also for the legal requests.[2]

Main Objectives of DNA Profiling/ Finger Printing Include:

- Identifying victims
- Associating body parts
- Identifying criminals.[3]

Historic Review

In 1985, **Jeffreyset**[4] al used radioactive probes to identify mini satellites (highly variable regions of DNA) to describe the pattern of the individual. These hyper-variable loci have tandem repeat of nucleotide succession. As reported to their size, they are named as variable number of tandem repeats (VNTR) or mini satellites which consists 9–80 base pairs or short tandem repeats (STRs). This revelation led to the use of DNA analysis in forensics for identifying human remains and solving disputed parentage affairs.[5] **Saiki et al**[6] introduced polymerase chain reaction (PCR) which was later followed and mechanized by **Mullis and Faloona**.[7] It includes amplification of interested sequences from very little quantities of DNA accessible.

Schwartz et al 1991[8] under variable environmental conditions isolated very high molecular DNA from dental pulp. Genomic dot blot hybridization was performed for identifying sex by **Pötsch et al** 1992.[9] In his study, he obtained a total genomic DNA from a dental sample, which, ranged between 6 µg and 50 µg.

In 1995 **Sweet et al**[10] identified a human remain from DNA that was Isolated from an un-erupted, preserved third molar. **Tsuchimochi,** et al., 2002[11] extracted pulpal DNA by incinerating extracted teeth at temperatures of 100°C, 200°C, 300°C, 400°C and 500°C for 2 min to conduct PCR analysis on them. No PCR product was produced for samples that were incinerated above 400°C whereas samples incinerated for up to 300°C could be amplified. The DNA was isolated using Chelex 100 chelating resin.

In 2003 **Malaver and Yunis**[12] evaluated in their study that the pulp produced the strongest PCR amplification signal when compared to dentin and cementum.

Principle for DNA Fingerprinting

The gene that codes for a particular protein contributes for only 2–6 % of DNA while the remaining 95% are the junk or noncoding DNA. This junk DNA may be present as a single copy of spacer DNA or as multiple copies called repetitive DNA. The repetitive sequence exists as long or STRs. The differences in the mini satellite pattern that is detected by a probe along with stable inheritance forms the basis for DNA fingerprinting.[13]

DNA-types

- **Genomic DNA** – Teeth provide a good Source of Genomic DNA.[20] They are within the nucleus of the cell.[14]
- **Mitochondrial DNA** – Used when DNA sample obtained is insufficient or degraded[10]

Stages in DNA Extraction

- Cell membrane rupture
- Denaturation of proteins using chelating agents and inactivation using proteinases DNA extraction. Most commonly.
- DNA is extracted by organic method (phenol) or by Chelex 100 (Bio Rad Laboratories, Inc), FTA paper (WhatmanInc, Clifton, NJ) or isopropyl alcohol.[1]

Methods of Running a DNA Fingerprint

DNA profiling or fingerprinting discloses the genetic makeup of a person. Teeth provide a remarkable source of DNA as they remain virtually unaffected by environmental assaults. Proper DNA extraction and quantification are needed to perform a successful analysis.

The Different Ways of Running A DNA Fingerprint are as Follows:

Restriction fragment length polymorphism method

After the evidence is collected from the crime scene, DNA is extracted . A special enzyme (restriction endonuclease) that acts as molecular scissors is used , DNA is grind into fragments at sites that are not found with in the tandem repeat sequence. The chopped fragments have VNTR of varying lengths.15 Gel electrophoresis is done to apart the cut fragments based on their size. Southern Blot (transferring the fragments to a nitrocellulose filter) is then performed, and a radioactive probe is used to evaluate the DNA. Restriction fragment length polymorphism detects the repeated sequences by defining a specific pattern to the VNTR, which forms the DNA fingerprint of a person. Restriction fragment length polymorphism (RFLP) requires immense quantities of DNA and requires long waiting time to obtain results.[16]

Polymerase Chain Reaction

Obtainable DNA is amplified to carry out the analysis using a special enzyme and DNA primers, which are accurate for human DNA, and the results remain unaffected even if bacterial DNA is present in the sample. The principle of PCR is the ability of DNA to replicate/duplicate itself. When the strands of DNA unwind during duplication, the primer is employed to amplify specific segments. After few hours, DNA is amplified to about 109 times the original amount and the reaction runs through 30 cycles.[17] Amplicons are the products of amplification, which are then detached by electrophoresis. PCR is used for evaluating VNTR, particularly the frequencies of STR loci. To determine the quantity of male or female DNA in a mixed sample, as in sexual assault cases, real-time PCR or quantitativen PCR was developed..

Short Tandem Repeat Typing

It is a commonly and routinely used marker in forensics. STRs have a high power of individual discernment because of their high standards of polymorphic informative content. The non overlapping size of the alleles from different contributors serves to distinguish them. Currently, they are detected by fluorescent detection methods using capillary or gel electrophoresis and even by ABI gel-based DNA sequencers while earlier works on detection of DNA involved silver-stained polyacrylamide gels. Used in paternity testing as each individual has some STRs inherited from father and some from the mother.18 They are hyper-variable regions that show repetitions of fragments having 2–7 base pairs.[2] It helps in identifying victims of mass calamities from even old remains.[19] To serve as the standard for the combined DNA index systems (CODIS), Federal Bureau of investigation has chosen 13 definite STR loci[20] which are together known as CODIS markers and the sex identifying amelogenin marker. A number of commercial kits are available that amplify the 13 core loci and amelogenin.

Analysis of Mitochondrial DNA

When the sample cells lack nucleus, DNA is extracted from mitochondrion. **Silva & Passos** in 2002 stated that mtDNA analysis can be used for ancient tissues like bone, hair and teeth, where analysis of nuclear DNA cannot be

done.[21] High molecular weight mtDNA are obtained from teeth, especially in degraded remains.[11] Every child has the duplicate mtDNA as its mother because mitochondrion of the embryo is from the mother's egg while genomic DNA is from father's sperm. It is thus an important tool in identifying missing persons by comparing mtDNA of unidentified remains with that of a possible maternal relative.[18] The technique is expensive as it is performed by direct sequencing of nitrogenous bases and provides limited data as it is primarily matrilineal.

Analysis of Y Chromosome

This type of analysis involves targeting of polymorphic regions of the Y chromosome (Y-STR) using primers. As Y chromosome is passed to the son from his father, analysis of markers on the chromosome helps in sketching relationships among males[22].

Single nucleotide polymorphism

They are variations that occur when a nucleotide sequence is altered. E,g : An SNP may change the nucleotide sequence AAGGCTAA to ATGGCTAA. Provides valuable information on descent, sex, evolution and it is highly automated.[24] Their advantage is that they can identify highly degraded DNA fragments.

The Recent Technologies in Genetic Identification

Microarray Technique

The polynucleotide of the target are amalgamate to high-density microarrays containing several thousand oligonucleotides immobilized on chips or beads[25] Many commercial platforms are available for SNP analysis. E,g : Affymetrix and Illumina. DNA analysis using this technology is used in forensic testing for sequencing and resequencing, paternity testing, SNP genotyping and identification of the individual.[26]

Next generation Genome Sequencing

Next generation genome sequencing permits analysis of several hundred loci or even the entire genome by producing enormously parallel sequencing.[3,27] Amplification or cloning of the sequenced DNA fragments is automated and provided with a reading process. Next generation genome sequencing platforms available are Illumina genome analyzer, Roche 454 genome sequencer and ABI Sequencing by Oligonucleotide Ligation and Detection. NGS permits analysis of copy number variants (CNVs) and structural rearrangements.

Next generation genome sequencing can be used for both genome and transcriptome analysis. In genome analysis, it permits the high-quality variant calling for SNPs, insertions and deletions, and allows the analysis of CNVs and other structural rearrangements.[23]

Conclusion

DNA fingerprinting is getting Vast and more universally accepted with time. Since no one can vary their DNA sequence after leaving it at the crime scene and because it is hard to prevent leaving one's DNA at the crime scene, DNA analysis is arguably the greatest forensic tool used in forensics of the three main kinds of DNA fingerprints, RFLP, VNTR and STR, the most commonly used is the STR. Restriction fragment length polymorphism and VNTR require alot of DNA, which is generally very difficult to find at the **Forensic** scene and often the DNA fragments being analyzed are too long to amplify via PCR. STR, on the other hand, uses short sections of DNA, which are perfect for running a PCR. There is an exponential increase of the volume of DNA, thus making it easier to run tests on small samples of DNA. Additionally, STR analysis does not require the hybridization to a DNA probe, which would have been time-consuming. Forensics has taken a large step using DNA to solve crimes, which were unsolvable in the past. As DNA is individualized, it is extremely rare that the DNA will match more than one person on this planet. This has allowed DNA evidence to be accepted in the court room.

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